Veterinär-Anatomisches Institut der Vetsuisse-Fakultät Universität Zürich

Direktor: Prof. Dr. A. Boos
Arbeit unter der Leitung von Prof. Dr. P. Wild

Effect of estrogens on parathyoid hormone secretion in cattle

Inaugural-Dissertation

zur Erlangung der Doktorwürde der
Vetsuisse-Fakultät Universität Zürich

vorgelegt von

Yvonne Sigrist
Tierärztin
von Greifensee, ZH

genehmigt auf Antrag von

Prof. Dr. P. Wild, Referent
Prof. Dr. R. Thun, Korreferent

Zürich 2009

Für meine Mutter

und in Erinnerung

an meinen Vater

Table of content

1 Abstract

Cell organelles concerned with parathyroid hormone secretion were shown to become enlarged 6 to 10 hours after application of estrogens to rats, suggesting that estrogens may stimulate parathyroid hormone secretion. To test whether this assumption is correct we treated cattle with estrogens and collected blood samples in 1 hour intervals for the first 10 hours, and thereafter in 2 hours intervals up to 24 hours. Analysis of plasma parathyroid hormone by radioimmunoassay revealed increments of parathyroid hormone that were mostly not negatively correlated with plasma calcium concentration. The responses to application of estrogen varied enormously considering magnitude and onset of alterations in parathyroid hormone, estrogen and calcium concentration. The data strongly suggest that estrogens have the ability to stimulate parathyroid hormone secretion via a genomic pathway because of the presence of estrogen receptors in bovine parathyroid cell nuclei detected by immunolabeling.

2 Introduction

2.1 PTH

The concentration of plasma parathyroid hormone (PTH) is inversely related to the concentration of plasma calcium (Arnaud and Tsao, 1969; Care et al., 1966; Ramberg et al., 1967; Sherwood et al., 1966; Sherwood et al., 1968). PTH synthesis and PTH release are mainly controlled by calcium via a unique negative feedback mechanism (Habener and Potts, 1976). Though parathyroid secretory activity is largely controlled by calcium, magnesium plays also an essential role (Sherwood, 1970; Sherwood et al., 1970). Many other cationic factors, e.g. manganese (Johansson et al., 1988), sodium, potassium (Brown et al., 1987), lithium, aluminum (Cournot-Witmer and Plachot, 1990), fluoride and chloride (Brown, 1982; Cannata et al., 1988; Shoback and McGhee, 1988), are involved in modulation of PTH secretion. Furthermore, non-ionic factors such as β-adrenergic catecholamines, prostaglandins E_2, serotonin, prolactin, dopamine and glucagon (Brown, 1982; Cannata et al., 1988; Habener, 1981) were shown to stimulate PTH secretion whereas α-adrenergic catecholamines, somatostatin and prostaglandines F_2 were reported to suppress PTH secretion (Brown, 1982).

2.2 Estrogen

Estrogens are also involved in PTH secretion. However, diverging opinions exist wether estrogens modify PTH secretion directly (Duarte et al., 1988; Greenberg et al., 1987; Naveh-Many et al., 1992; Shen et al., 2000; Silver et al., 1994) or indirectly via hypocalcaemia resulting from inhibition of calcium resorption from bone (Gallagher et al., 1980; Prince et al., 1991; Saxe et al., 1992; Shen et al., 2000; Vincent et al., 2003). In vitro studies on isolated parathyroid cells revealed clear evidence that E_2 has a direct effect on PTH secretion (Greenberg et al., 1987).

2.3 Regulation of PTH synthesis and release

PTH is synthesized on ribosomes of the rough endoplasmic reticulum (RER), packaged in the Golgi complex into secretory granules for storage and transportation to the cell periphery where it is released by exocytosis (Habener, 1981). Autoradiographic studies showed that the whole process takes about 30 min (Habener et al., 1979). PTH release is modulated within seconds (Blum et al., 1974). To modulate PTH synthesis hours are required (Habener, 1981). Administration of β-estradiol-3-benzoate (E_2) to rats provoked an initial membrane shift from the Golgi complex and secretory granules to the plasma membrane in parathyroid (PT) cells within 90 minutes suggesting that PTH release has been provoked by estradiol (Wild et al., 1989) because a similar membrane shift was apparent in PT cells stimulated by depression of serum calcium (Wild et al., 1982). In addition, a transient depression of serum calcium resulted in drastic enlargement of the compartments concerned with PTH synthesis after more than 6 hours suggesting increment of capacity for PTH production (Wild et al., 1985). Similarly, compartments concerned with PTH secretion were also substantially enlarged 6 hours after E2 treatment suggesting that estradiol modulates also the capacity for PTH synthesis, packaging and storage. (Wild et al., 1989).

2.4 Aim of the study

On the basis of the behavior of membranes concerned with PTH secretion we tested whether estradiol stimulates, indeed, PTH release within minutes. Therefore, we treated cattle with E_2 and took blood samples every 15 min for analysis of E_2, PTH and calcium. To evaluate whether estradiol influences PTH synthesis cattle were treated with 4 consecutive injections of E_2 in intervals of 2 h because a minimal exposure time for 6 h was required to stimulate membrane synthesis necessary for enlargement of the PTH synthesis machinery (Wild et al., 1989). The data suggest that E_2 may have an effect on PTH synthesis probably via a genomic

pathway as immunolabeling for E_2 receptors in PT cells was positive. The short-term effect on PTH release could not be convincingly demonstrated.

3 Materials and methods

3.1 Experimental Design

Six 3 to 4 years old non lactating cattle in the anestrous phase of the Swiss brown breed kept on a diary farm were used for this study. 0.01 mg E_2/kg body weight (beta-estradiol-3-benzoate; Streuli, Uznach, Switzerland) was administered by intramuscular injection into the gluteal muscle at 0, 2, 4, and 6 h as it had been shown that it takes at least 6 h of E_2 exposure to activate the machinery for PTH synthesis and packaging (Wild et al., 1989). Blood samples were taken through a catheter (Becton & Dickinson, Rutherford, NY, USA) implanted into a jugular vein 24 h prior to the first E_2 administration. Samples were taken every hour from 0 to 10 h of the experiment, and then in intervals of 2 h up to 24 h. For controls, 4 cattle received 4 times the vehicle substance. Blood sample were taken in 15 min intervals during the first 2 h and then in same intervals as described. In addition, these animals received a single dose of E_2 at 24 h. Blood samples were taken in intervals of 15 minutes during 2 hours after E_2 administration in order to determine a possible short term effect of E_2 on PTH release. Blood was collected in 20 ml syringes containing heparin (B. Braun, Melsungen, Germany) and centrifuged at low speed. 1.5 ml aliquots of plasma were frozen within 15 minutes after collection and stored at -20°C.

3.2 Determination of plasma calcium concentration

The plasma calcium concentration was determined employing a spectroscopy calcium test (Böhringer, Mannheim, Germany) according to the o-kresolphtalein-complex-method without withdrawal of proteins (Ray Sacar B.C. et al. 1967). The analysis was checked with a control serum (Precinorm U, Böhringer, Mannheim, Germany).

3.3 Estimation of parathyroid hormone and estradiol

Plasma PTH concentration was determined by radioimmunoassay using kits from Nichols Institute Diagnostics (Chantilly, Virginia, USA). In a preliminary experiment we checked the efficiency of the method for bovine PTH after induction of hypocalcemia. 1.25 mg/kg body weight ethylene-glycol-tetra acetic-acid (EGTA, Fluka, Buchs, Switzerland) was infused into a jugular vein at a speed of 0.5 ml/min for 7 minutes according to (Blum et al., 1974). 10 blood samples were taken from the other jugular vein in 15 min intervals for determination of calcium concentration and PTH. The detection limit was 12 ng/ml. Plasma concentration of E_2 was determined by radioimmunoassay using kits from Immunotech (Marseille, France). The detection limit was 0.45 pg/ml. Data were analyzed by a multiple t test using the Prisma software.

3.4 Immunocytochemistry

Bovine PT glands were harvested at the local abattoir. They were immersed into ice chilled 0.1 M Na-phosphate, pH 7.2, for transportation to the laboratory for fixation with 2% paraformaldehyde at room temperature for 30 min. After freezing cryo-sections were prepared and immunostained for E-receptors using monoclonal antibodies (Abbott Laboratories,

Abbott Park, Illinois, USA). For controls, samples from the endometrium of the same cattle were used. For controls samples of the uterus of the same animals were used.

4 Results

Administration of E_2 resulted in marked alterations of plasma PTH concentration that varied considerably among animals considering magnitude and time of onset. Statistical analysis of PTH and of plasma calcium concentration thus did not reveal any significant differences. Therefore, data of individual animals were compared and described in detail.

4.1 Alterations of E_2, PTH and calcium from 0 to 10 hours

The compartments concerned with PTH synthesis have been shown to be drastically increased 6 to 12 after an initial depression of serum calcium (Wild et al., 1984) or after 4 consecutive applications of E_2 (Wild et al., 1989) in rat PT cells suggesting enhancement of the capacity for PTH synthesis and possibly of PTH release. To verify whether or not estradiol influences PTH synthesis we administered E_2 four times in intervals of 2 h. As shown in Fig. 1 E_2 increased dramatically from 4 to 150-200 pg/ml within 3 hours in 2 animals (C1 and C2). E_2 fluctuated at this level in C1 for about 10 h whereas in C2 it declined to 80 pg/ml at 4 h followed by a steady increase to 110 pg/ml at 10 h. E_2 rose continuously in C3, rose drastically in C5 from 5^{th} to 6^{th} h but rose slowly in C4 and C6 to 57 and 20 pg/ml, respectively. The heterogeneity in response to E_2 administration considering time and magnitude suggests great variations in PTH concentrations if E_2 has has an effect on PTH secretion. Indeed, in C1 PTH increased drastically to a first peak at 3 h and to a maximum of 167 ng/ml at 8 h. In C2 PTH rose only to 56 ng/ml at 3 h, and to 140 ng/ml at 7 h after the first injection of E_2. PTH peaked at 5 h in C3 (60 ng/ml) and C6 (88 ng/ml), and at 8 h in C5 starting to rise at 7 h after the first application of E_2. C4 showed no response. Interestingly, PTH declined (except in C1) during the first 2 hours but rose slightly from the 2^{nd} to the 3^{rd} h.

To clarify whether the increments of PTH are a direct effect of E_2 onto the secretory activity of PT cells we compared the PTH peaks with alterations of plasma calcium. In C1 and C2 all PTH peaks were positively correlated with [Ca^{++}]. The PTH peak at 5h in C3 was inversely related to changes in [Ca^{++}]. However, the decrease of [Ca^{++}] by only 0.2 mmol/ml does not justify the assumption that it was responsible for the rise in PTH of more than 40 ng/ml at 5 h. Plasma [Ca^{++}] in C5 dropped by 1.25 mmol/ml between 6 and 7 h without concomitant rise in PTH. Therefore, the minor decrease of 0.2 mmol from 7 to 8 h is unlikely to be responsible for the rise in PTH of 90 ng/ml especially since [Ca^{++}] remained constant for the next hour whereas PTH drastically decreased. The rise in PTH of 60 ng/ml from 4 to 5 h in C6 correlated with a decrease in [Ca^{++}] of 0.5 mmol/mml whereas the PTH peak at 7 h was positively related to [Ca^{++}]. These data strongly suggest that changes in [Ca^{++}] were not primarily responsible for the marked increments of PTH and that E_2 played the major role in regulation of PTH secretion.

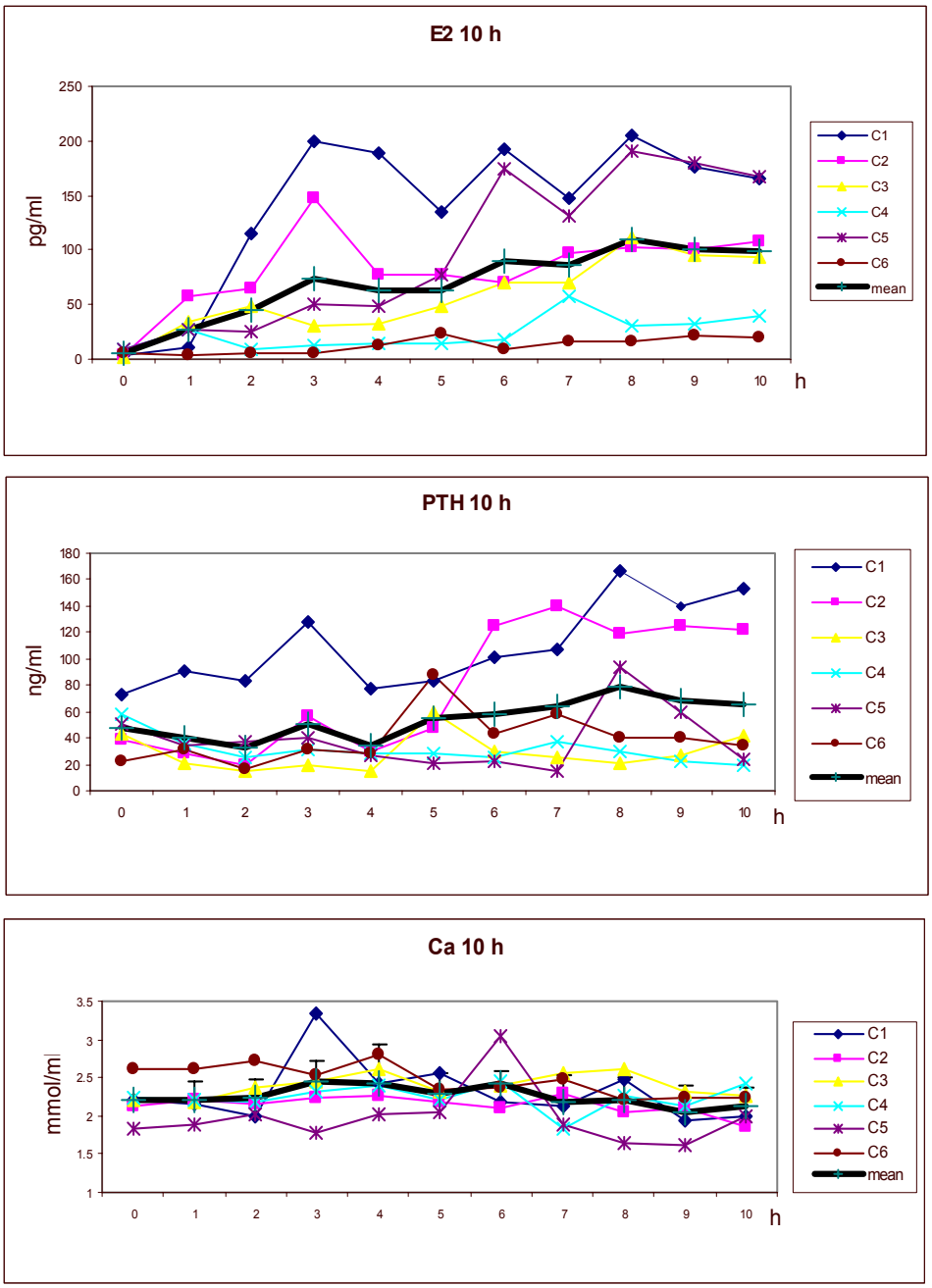

Fig. 1 Plasma E_2, PTH and Ca^{++} concentration of blood samples taken form 0 to 10 h after administration of E_2.

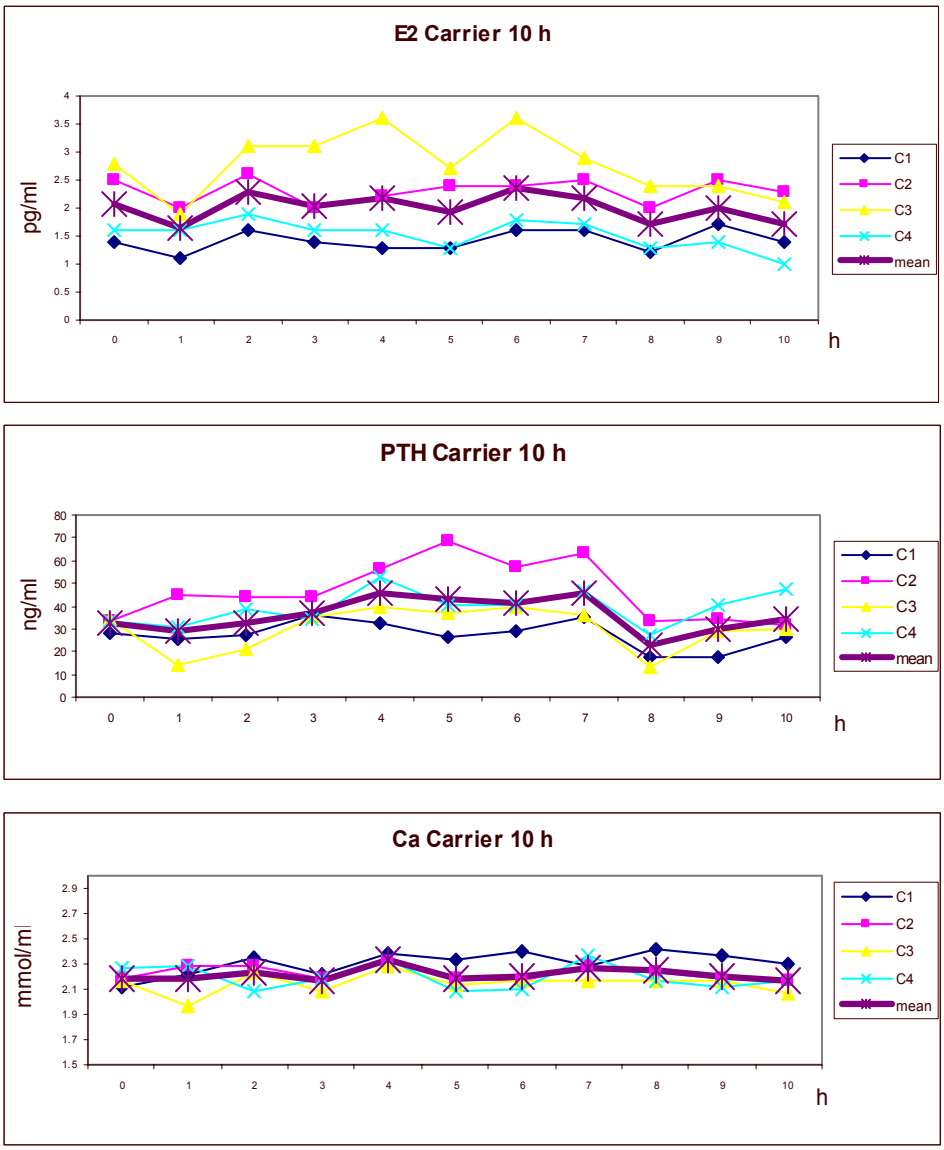

Fig. 2 Plasma E_2, PTH and Ca^{++} concentration of blood samples taken form 0 to 10 h after administration of the carrier substance.

For controls we injected the carrier solution without E_2 to 4 animals (Fig. 2). Interestingly, PTH rose from 45 ng/ml at 3 h to 70 ng/ml at 5 h in C8. The rise from 3 to 4 h was positively correlated with plasma calcium as well as with E_2. However, the variations were small. In C9

PTH decreased within the first h but increased to 40 ng/ml at 4 h. This increase was also positively related to an increase in calcium and E_2 whereby the increase in E_2 was more pronounced than that of calcium. These data suggest that the increase in PTH was rather due to physiological changes in E_2 than by changes in calcium concentration. Alternatively, it might have been caused by other factors such as stress. All other data were within normal ranges suggesting that the rise of PTH in E_2 treated animals was a response of PT cells to E_2.

4.2 Alterations of E_2, PTH and calcium from 10 to 24 hours

If the idea is correct that enlargement of the machinery of PTH secretion as detected at 12 h after a single massive depression of calcium concentration (Wild et al., 1985) or after administration of E_2 (Wild et al., 1989) in rats it will be expected that plasma PTH concentration will stay at a high level or even further increase after 10 h of exposure to a stimulating agent. Determination of PTH (Fig. 3) revealed fluctuations in C1 and C2 reaching peaks at 14 h (C1: 160 ng/ml) and 16 h (C2: 180 g/ml), respectively. Then PTH declined in both animals to about 70 ng/ml at 24 h. PTH fluctuated between 20 and 50 ng/ml in C3 and C4. In C5 PTH started to rise after 18 h to 84 ng/ml whereas in C6 PTH started to rise at 20 h almost to 120 ng/ml. The marked depression of PTH in C1 and C2 at 12 h was negatively correlated with $[Ca^{++}]$ whereas the peaks at 14 h (C1) and 16 h (C2) were not. In C1 $[Ca^{++}]$ fluctuated between 2.6 and 2.5 mmol/ml from 12 to 18 h, and increased to 2.9 mmol/ml at 20 h. The increment of PTH in C1 from 12 to 14 h was followed by a drastic decrease to 84ng/ml at 16 h whereas there was only a minor decrease of PTH from 18 to 20 h at the time $[Ca^{++}]$ rose by 0.4 mmol/ml. In C2 PTH increased drastically from 12 to 16 h whereas $[Ca^{++}]$ increase from 14 to 18 h. In C5 $[Ca^{++}]$ increased from 1.7 mmol/ml at 12 h to 2.7 mmol/ml at 16 h, and rapidly decreased to 1.6. mmol/ml at 20 h. PTH increased slowly from 10 to 18 h but fast from 18 to 20 h. In C6 there were little changes in PTH between 10 and 20 h despite a

remarkable decrease of [Ca^{++}] from 16 to 18 h. However, PTH rose drastically from 20 to 22 h whereas [Ca^{++}] rose from 18 to 22 h. These data show that alterations in PTH were mostly not negatively related to changes in [Ca^{++}].

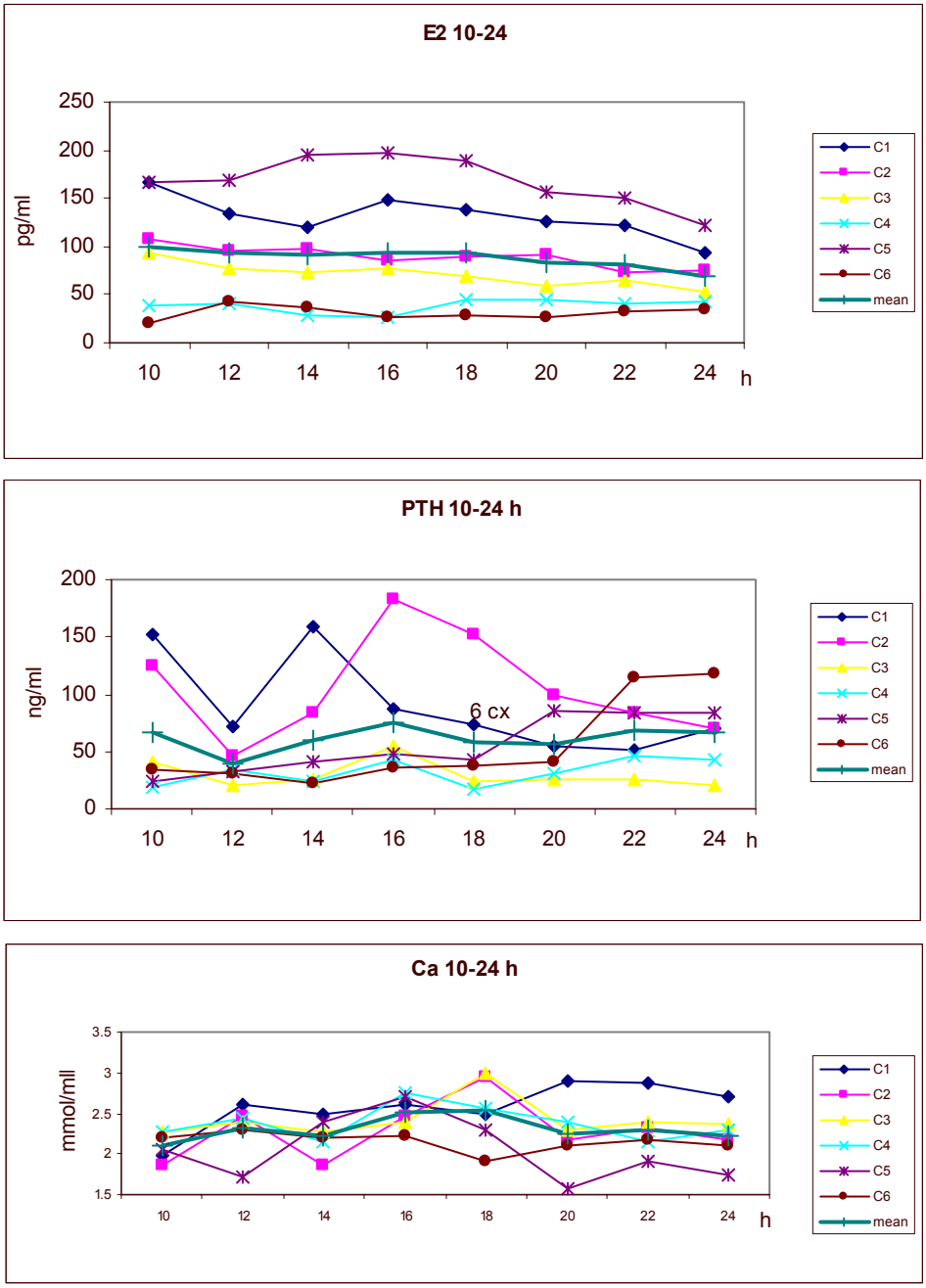

Fig. 3. Plasma E_2, PTH and Ca^{++} concentration of blood samples taken form 10 to 24 h in 2 hours intervals after administration of E_2.

Administration of the carrier substance had no obvious effect between 10 and 24 h (Fig. 4). Variations in PTH, [Ca^{++}] and E_2 were low. Changes in PTH were often but not always negatively correlated with [Ca^{++}] which varied maximally within a range of 0.2 mmol/ml in a given animal. The range of E_2 did not exceed 1 pg/ml and that of PTH was maximal 20 ng/ml in a given animal.

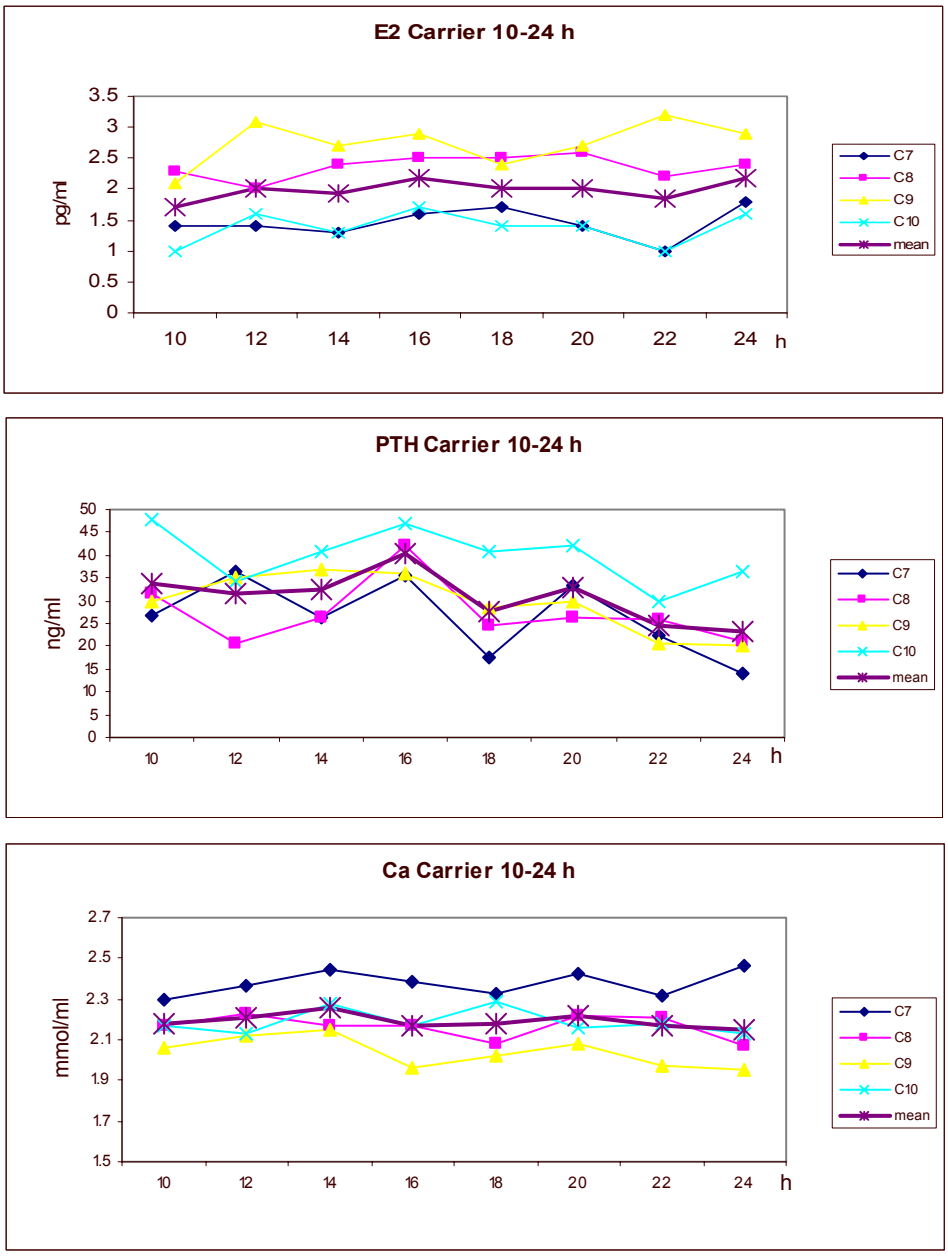

Fig. 4 Plasma E_2, PTH and Ca^{++} concentration of blood samples taken form 10 to 24 h in 2 hours intervals after administration of the carrier substance.

4.3 Short-term Alterations of E_2, PTH and calcium

To clarify whether E_2 may have a short-term effect on PTH release, a single dose of E_2 was injected to the animals 24 hours after treatment with the carrier substance. Analyses of blood samples taken in intervals of 15 min revealed a rise of PTH at 15 min in 2 of the 4 animals (Fig.5) that were accompanied by a decrease in [Ca++]. However, the rise in PTH was more than 20 ng/ml in C8 and C9 whereas the decrease in [Ca^{++}] was less than 0.1 mmol/ml. PTH peaked also at 75 min in C7, C8 and C9. The rises in PTH from 60 to 75 min were positively correlated with [Ca^{++}] in C8 and C9. The rise of PTH from 60 to 120 min in C8 can also not be attributed to decrements in [Ca++].

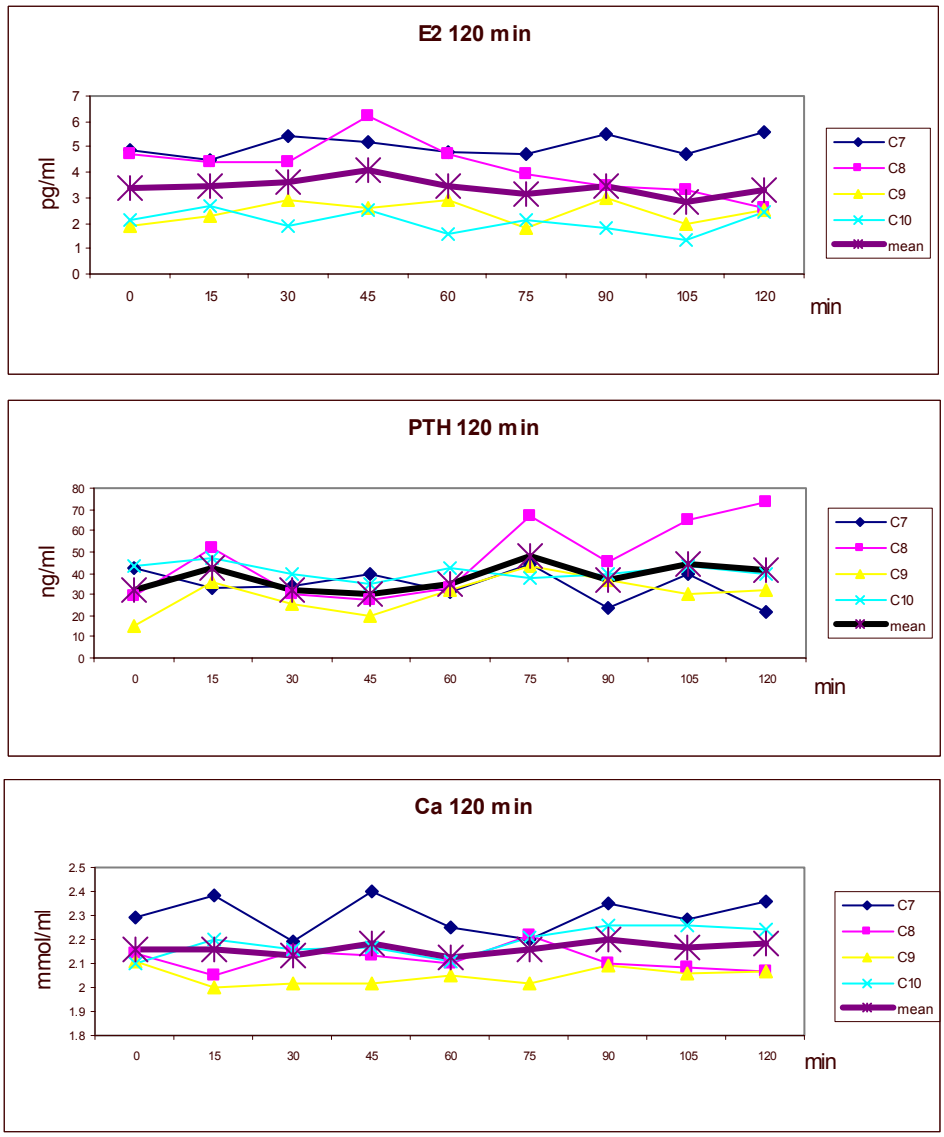

Fig. 5 Plasma E_2, PTH and Ca^{++} concentration of blood samples taken form 0 to 120 min in 15 minutes intervals after administration of E_2.

Interestingly, PTH peaked at 45, 75 and 105 min after administration of the carrier substance in C8 (Fig. 6). The peaks at 45 and 105 min were not negatively correlated to [Ca++]. Variations of PTH in the other animals were within normal ranges but not always negatively correlated with [Ca++]. These data do not show convincingly that E2 may modulate PTH release.

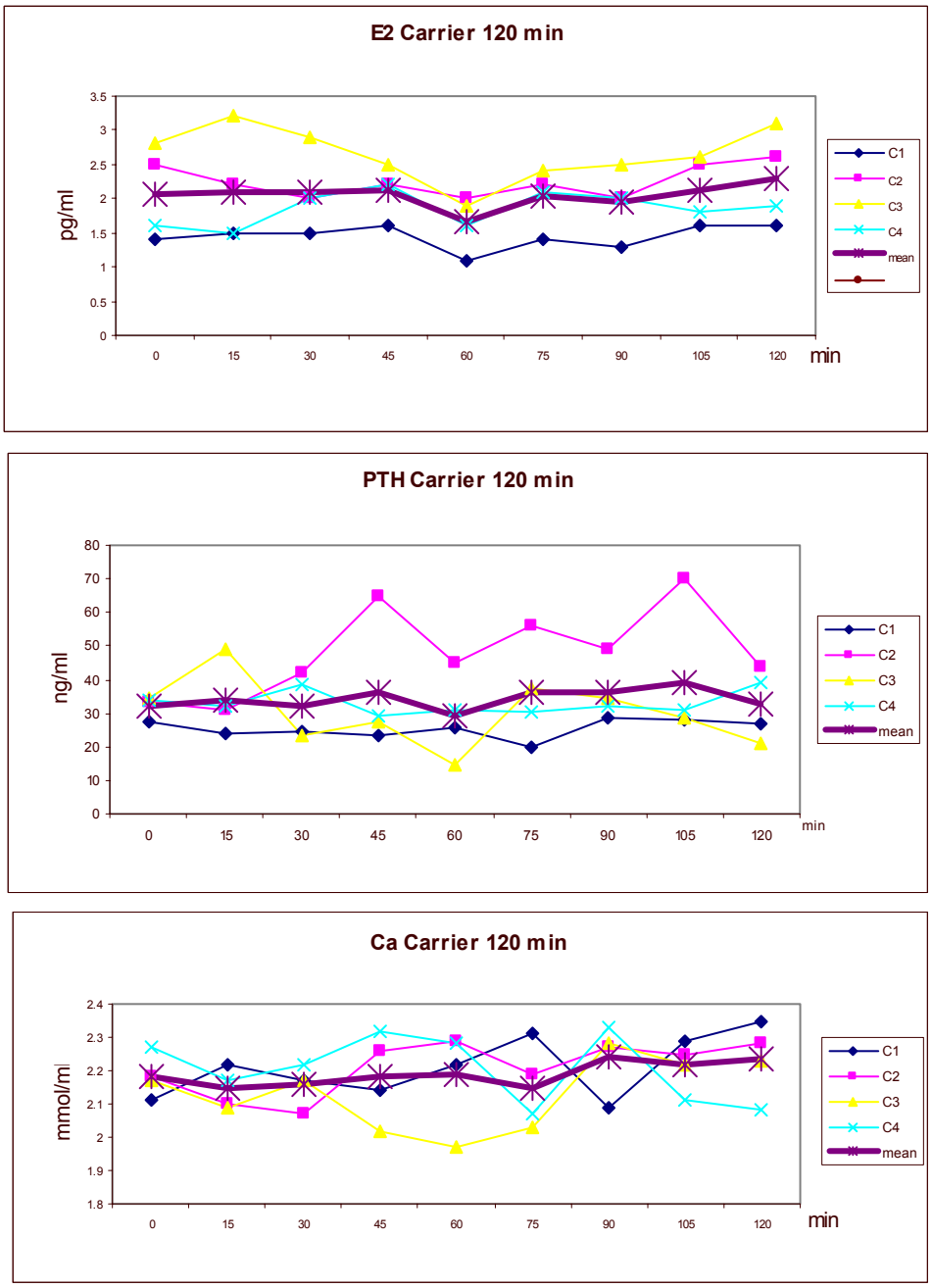

Fig. 6 Plasma E_2, PTH and Ca^{++} concentration of blood samples taken form 0 to 120 min in 15 minutes intervals after administration of the carrier substance.

4.4 E_2 receptors are present on parathyroid cell nuclei

The increments in PTH after 5 h of the first administration of E_2 are rather due to E_2 than to decrements of $[Ca^{++}]$ because variations in plasma $[Ca^{++}]$ were small and not always negatively correlated to rises in PTH. The late response of PTH secretion to E_2 suggests a genomic pathway involving E_2 receptors (Naveh-Many et al., 1992) at least after application of estradiol. Immunolabeling of bovine PT tissue harvested at a local abattoir yielded faint staining of PT cell nuclei for E_2 (Fig. 7) as was claimed to be the locus in intact cells (King and Greene, 1984; Welshons et al., 1984).

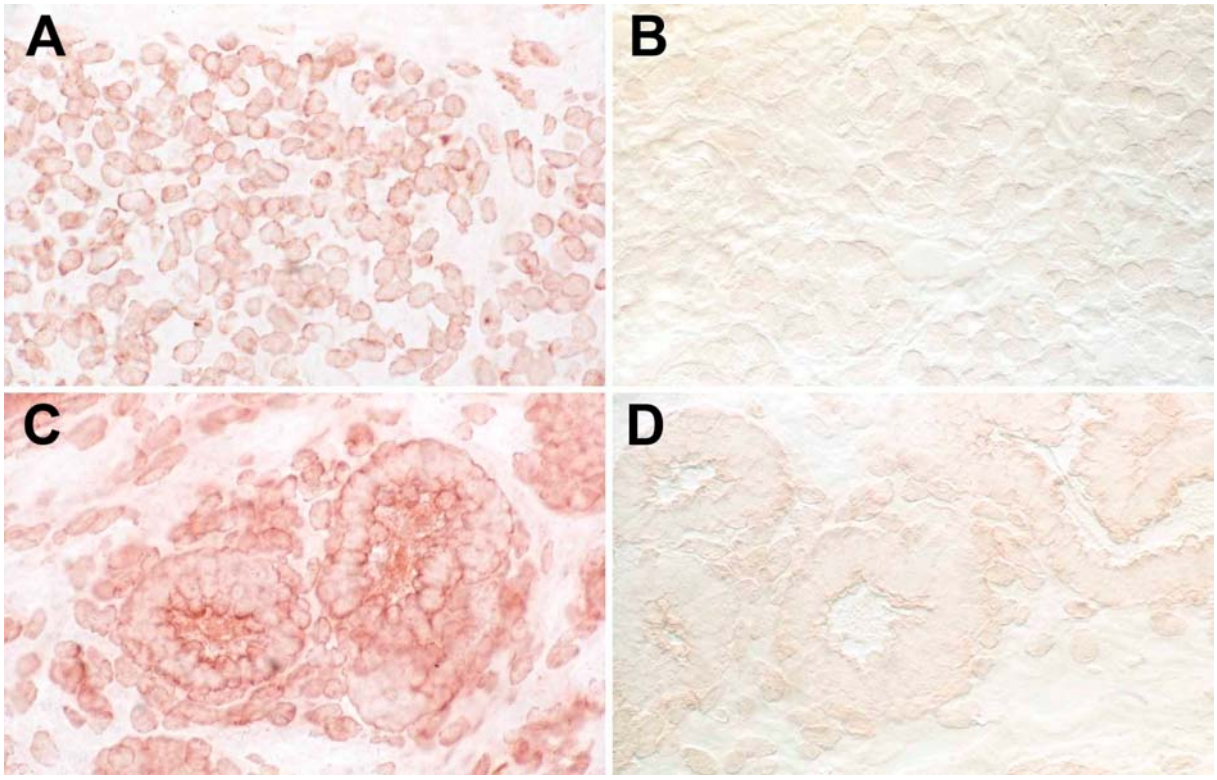

Fig. 7 Immunostaining of parathyroid cells (A) and endometrial glands (C) with monoclonal antibodies against estrogen receptors. For negative controls primary antibody was avoided in cryo-sections of parathyroid (B) and endometrial glands (D)

5 Discussion

This study was designed to evaluate the effect of estrogens on parathyroid PT secretory activity on the basis of results obtained in studies on membrane dynamics in rat PT cells that showed substantial response to E_2 after administration in 4 consecutive steps in intervals of 2 h (Wild et al., 1989). Analysis of blood collected from cattle treated with 4 consecutive injections of E_2 clearly revealed that E_2 has the ability to stimulate PTH secretion as the enlargement of the machinery concerned with PTH secretion had suggested. The response to E_2 administration varied considerably among the animals in respect to onset and magnitude. Administration of β-estradiol-3-benzoate, a formulation designed for intramuscular injection, resulted in a rapid rise in plasma E_2 starting after 2 h and in a first increase of plasma PTH at 3 h followed by a second increase starting at 5h in C1 and C2. The increments were not related to decrements in $[Ca^{++}]$ (Fig. 8) and are hence considered likely to be the result of a stimulating effect of E_2 on PT secretory activity. Increments of PTH in the other animals were not as dramatic as in C1/C2 and occurred later. Increments of PTH in C5 and C6 were less pronounced, occurred later and were not or not clearly related to decrements in $[Ca^{++}]$. C3 and C4 showed little response on PTH. Interestingly, $[Ca^{++}]$ increased substantially between 2 and 4 h in C1 and C3, but later in the other animals. Most $[Ca^{++}]$ increments were not negatively related with PTH, except in C3. The early rises in $[Ca^{++}]$ are considered likely to be the result of a hypocalcemic effect of estrogens (Prince et al., 1990), which in turn results from estrogen-mediated inhibition of bone resorption (Gallagher et al., 1980; Riggs and Melton, 1986; VanHouten and Wysolmerski, 2003). PTH was considered to stimulate osteoblast function after pretreatment with estrogens (Nasu et al., 2000). On the other hand, estrogens stimulate gene expression and production of osteoprotegerin in osteoblastic cells that is supposed to play a major role in the antiresorptive action of estrogens in bone (Hofbauer et

al., 1999). It has also been shown that PTH was significantly higher during the estrus in weanling rats (Epstein et al., 1996).

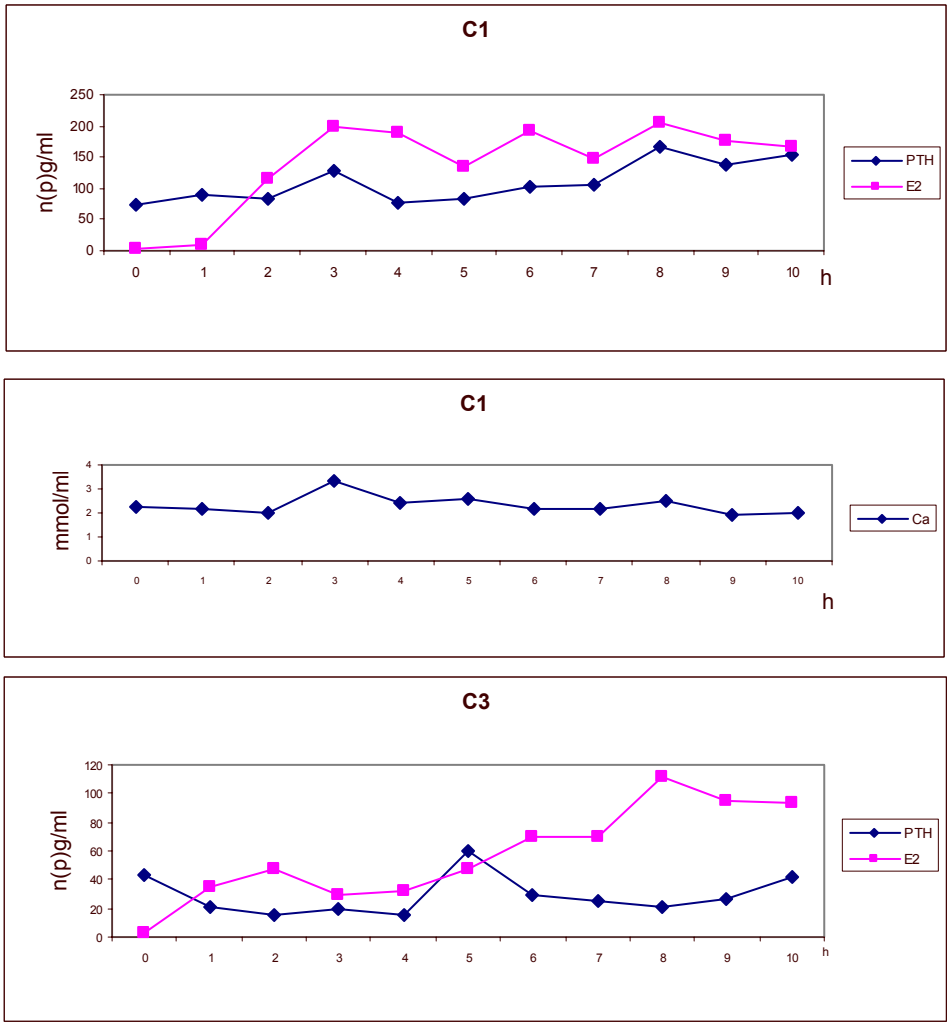

Fig. 8 Plasma E_2, PTH and Ca^{++} concentration of blood samples taken form 0 to 10 h of cattle C1 and C3 after administration of E_2.

Despite the heterogeneity of the responses there is little doubt about a direct stimulating effect of E_2 on PT secretory activity. The presence of estrogen-receptors on PT cell nuclei suggests a genomic pathway (McCarty et al., 1983; Migliaccio and Marino, 2003; Moggs and Orphanides, 2001). The conclusion, however, whether or not E_2 may modulate immediately PTH release cannot be deduced from the data obtained from blood samples collected in 15 min intervals because of the heterogeneity in responses, the small increases of PTH and because of decrements in $[Ca^{++}]$ – although very low – accompanying PTH increments. A short but distinct depression of $[Ca^{++}]$ was shown to be sufficient to induce a rapid release of PTH resulting in a drastic increase of PTH within 7 min (Blum et al., 1974). On the other hand, release of PTH was also shown to be stimulated by estrogens in isolated human PT glands (Duarte et al., 1988) and bovine PT tissue culture (Greenberg et al., 1987). However, estrogens were reported to have no direct effect on PTH secretion in post menopausal women 3 days after transdermal application (Vincent et al., 2003).

Whether E_2 may modulate PTH release has probably a much less physiological significance than its ability to stimulate PTH synthesis. The ability of E_2 to stimulate PTH synthesis may be of importance in bone remodeling, and consequently, in the onset of e.g. osteoporosis in humans. Alternatively, it might play a role in the pathogenesis of hypocalcemia in cattle. E_2 increases prior to parturition (Eissa and el-Belely, 1990) that may or may not sufficiently stimulate PTH synthesis before the decrement of serum calcium due to the high demand provoked by producing milk. The ability of E_2 to stimulate PTH synthesis may also be used to prevent the onset of post-parturient hypocalcemia. Stimulation of PT secretory activity prior to emergencies is extremely important because the storage capacity of PTH is low due to the low numbers of secretory granules in PT cells (Setoguti et al., 1995).

The heterogeneity in the response to administration of E_2 had probably different reasons. One is the different absorption rate of substances injected intramuscularly. Beta-estradiol-3-benzoate prepared as a crystalline suspension in water is rapidly absorbed after intramuscular administration and rapidly metabolized, the half-life time being 50 min according to the manufacturer (Streuli, Uznach, Switzerland). The fact that in 2 animals E_2 increased only slowly and only for a few picograms suggests that absorption was for one or the other reason disturbed. Another reason might be the number of estrogen receptors present in PT cells. The weak staining detected on PT cell nuclei only without counterstaining using an interference microscope indicate that the number of estrogen receptors in PT cells are low. Other investigators could not show estrogen receptors at all in bovine and human parathyroid tissue (Prince et al., 1991; Saxe et al., 1992). It had been shown that the number of estrogen receptors varies, and that it increases after administration of estrogens in fish (Sabo-Attwood et al., 2007), in mammary tumor cells (Hannouche et al., 1982), and in PT cells and C-cells (Naveh-Many et al., 1992).

Taken together, the data show that estrogens have the ability to stimulate PTH secretion. The diverse response in PTH secretion after administration of estrogen may be crucial e.g. in the onset of post-parturient hypocalcemia.

6 References

Arnaud, C.D., and H.S. Tsao, 1969. Porcine calcitonin. Simple procedure for isolation in high yield. Biochemistry 8: 449-56.

Blum, J.W., J.A. Fischer, D. Schwoerer, W. Hunziker, and U. Binswanger, 1974. Acute parathyroid hormone response: sensitivity, relationship to hypocalcemia, and rapidity. Endocrinology 95: 753-9.

Brown, E.M., 1982. PTH secretion in vivo and in vitro. Regulation by calcium and other secretagogues. Mineral & Electrolyte Metabolism 8: 130-50.

Brown, E.M., E.J. Watson, J.G. Thatcher, R. Koletsky, B.F. Dawson-Hughes, J.T. Posillico, and D.M. Shoback, 1987. Quabain and low extracellular potassium inhibit PTH secretion from bovine parathyroid cells by a mechanism that does not involve increases in the cytosolic calcium concentration. Metabolism 36: 36-42.

Cannata, J.B., J.B. Diaz Lopez, M.J. Fernandez Menendez, and M.J. Virgos, 1988. The parathyroid gland and aluminum overload: an overview. Contributions to Nephrology 64: 113-9.

Care, A.D., L.M. Sherwood, J.T. Potts, Jr., and G.D. Aurbach, 1966. Perfusion of the isolated parathyroid gland of the goat and sheep. Nature 209: 55-7.

Cournot-Witmer, G., and J.J. Plachot, 1990. Parathyroid glands in chronic aluminum intoxication. Ultrastructural Pathology 14: 211-9.

Duarte, B., G.K. Hargis, and S.C. Kukreja, 1988. Effects of estradiol and progesterone on parathyroid hormone secretion from human parathyroid tissue. Journal of Clinical Endocrinology & Metabolism 66: 584-7.

Eissa, H.M., and M.S. el-Belely, 1990. Sequential changes in plasma progesterone, total oestrogens and corticosteroids in the cow throughout pregnancy and around parturition. British Veterinary Journal 146: 24-9.

Epstein, E., J. Silver, G. Almogi, N. Livni, and T. Naveh-Many, 1996. Parathyroid hormone mRNA levels are increased by progestins and vary during rat estrous cycle. American Journal of Physiology 270: E158-63.

Gallagher, J.C., B.L. Riggs, and H.F. DeLuca, 1980. Effect of estrogen on calcium absorption and serum vitamin D metabolites in postmenopausal osteoporosis. Journal of Clinical Endocrinology & Metabolism 51: 1359-64.

Greenberg, C., S.C. Kukreja, E.N. Bowser, G.K. Hargis, W.J. Henderson, and G.A. Williams, 1987. Parathyroid hormone secretion: effect of estradiol and progesterone. Metabolism: Clinical & Experimental 36: 151-4.

Habener, J.F., 1981. Regulation of parathyroid hormone secretion and biosynthesis. Annual Review of Physiology 43: 211-23.

Habener, J.F., and J.T. Potts, Jr., 1976. Relative effectiveness of magnesium and calcium on the secretion and biosynthesis of parathyroid hormone in vitro. Endocrinology 98: 197-202.

Habener, J.F., M. Amherdt, M. Ravazzola, and L. Orci, 1979. Parathyroid hormone biosynthesis. Correlation of conversion of biosynthetic precursors with intracellular protein migration as determined by electron microscope autoradiography. Journal of Cell Biology 80: 715-31.

Hannouche, N., S. Samperez, M.R. Riviere, and P. Jouan, 1982. Estrogen and progesterone receptors in mammary tumors induced in rats by simultaneous administration of 17 beta-estradiol and progesterone. Journal of Steroid Biochemistry 17: 415-9.

Hofbauer, L.C., S. Khosla, C.R. Dunstan, D.L. Lacey, T.C. Spelsberg, and B.L. Riggs, 1999. Estrogen stimulates gene expression and protein production of osteoprotegerin in human osteoblastic cells. Endocrinology 140: 4367-70.

Johansson, H., R. Larsson, C. Wallfelt, J. Rastad, G. Akerstrom, and E. Gylfe, 1988. Calcium-agonistic action of Mn2+ in the parathyroid cell. Molecular & Cellular Endocrinology 59: 77-82.

King, W.J., and G.L. Greene, 1984. Monoclonal antibodies localize oestrogen receptor in the nuclei of target cells. Nature 307: 745-7.

McCarty, K.S., Jr., D.B. Lubahn, and K.S. McCarty, Sr., 1983. Oestrogen and progesterone receptors: physiological and pathological considerations. Clinics in Endocrinology & Metabolism 12: 133-54.

Migliaccio, S., and M. Marino, 2003. Estrogens and estrogen receptors: new actors in the plot of transcriptional regulation of genomic responses. Calcified Tissue International 72: 181-2.

Moggs, J.G., and G. Orphanides, 2001. Estrogen receptors: orchestrators of pleiotropic cellular responses. EMBO Reports 2: 775-81.

Nasu, M., T. Sugimoto, H. Kaji, and K. Chihara, 2000. Estrogen modulates osteoblast proliferation and function regulated by parathyroid hormone in osteoblastic SaOS-2 cells: role of insulin-like growth factor (IGF)-I and IGF-binding protein-5. Journal of Endocrinology 167: 305-13.

Naveh-Many, T., G. Almogi, N. Livni, and J. Silver, 1992. Estrogen receptors and biologic response in rat parathyroid tissue and C cells. Journal of Clinical Investigation 90: 2434-8.

Prince, R.L., I. Schiff, and R.M. Neer, 1990. Effects of transdermal estrogen replacement on parathyroid hormone secretion. Journal of Clinical Endocrinology & Metabolism 71: 1284-7.

Prince, R.L., D.T. MacLaughlin, R.D. Gaz, and R.M. Neer, 1991. Lack of evidence for estrogen receptors in human and bovine parathyroid tissue. Journal of Clinical Endocrinology & Metabolism 72: 1226-8.

Ramberg, C.F., Jr., G.P. Mayer, D.S. Kronfeld, G.D. Aurbach, L.M. Sherwood, and J.T. Potts, Jr., 1967. Plasma calcium and parathyroid hormone responses to EDTA infusion in the cow. American Journal of Physiology 213: 878-82.

Riggs, B.L., and L.J. Melton, 3rd, 1986. Involutional osteoporosis. New England Journal of Medicine 314: 1676-86.

Sabo-Attwood, T., J.L. Blum, K.J. Kroll, V. Patel, D. Birkholz, N.J. Szabo, S.Z. Fisher, R. McKenna, M. Campbell-Thompson, and N.D. Denslow, 2007. Distinct expression and activity profiles of largemouth bass (Micropterus salmoides) estrogen receptors in response to estradiol and nonylphenol. Journal of Molecular Endocrinology 39: 223-37.

Saxe, A.W., G.W. Gibson, I.H. Russo, and P. Gimotty, 1992. Measurement of estrogen and progesterone receptors in abnormal human parathyroid tissue. Calcified Tissue International 51: 344-7.

Setoguti, T., Y. Inoue, and P. Wild, 1995. The biological significance of storage granules in rat parathyroid cells. Microscopy Research & Technique 32: 148-63.

Shen, V., R. Birchman, D.D. Wu, and R. Lindsay, 2000. Skeletal effects of parathyroid hormone infusion in ovariectomized rats with or without estrogen repletion. Journal of Bone & Mineral Research 15: 740-6.

Sherwood, L.M., 1970. Magnesium ion and parathyroid function. New England Journal of Medicine 282: 752.

Sherwood, L.M., I. Herrman, and C.A. Bassett, 1970. Parathyroid hormone secretion in vitro: regulation by calcium and magnesium ions. Nature 225: 1056-8.

Sherwood, L.M., J.T. Potts, Jr., A.D. Care, G.P. Mayer, and G.D. Aurbach, 1966. Evaluation by radioimmunoassay of factors controlling the secretion of parathyroid hormone. Nature 209: 52-5.

Sherwood, L.M., G.P. Mayer, C.F. Ramberg, Jr., D.S. Kronfeld, G.D. Aurbach, and J.T. Potts, Jr., 1968. Regulation of parathyroid hormone secretion: proportional control by calcium, lack of effect of phosphate. Endocrinology 83: 1043-51.

Shoback, D.M., and J.M. McGhee, 1988. Fluoride stimulates the accumulation of inositol phosphates, increases intracellular free calcium, and inhibits parathyroid hormone release in dispersed bovine parathyroid cells. Endocrinology 122: 2833-9.

Silver, J., E. Moallem, E. Epstein, R. Kilav, and T. Naveh-Many, 1994. New aspects in the control of parathyroid hormone secretion. Current Opinion in Nephrology & Hypertension 3: 379-85.

VanHouten, J.N., and J.J. Wysolmerski, 2003. Low estrogen and high parathyroid hormone-related peptide levels contribute to accelerated bone resorption and bone loss in lactating mice. Endocrinology 144: 5521-9.

Vincent, A., B.L. Riggs, E.J. Atkinson, A.L. Oberg, and S. Khosla, 2003. Effect of estrogen replacement therapy on parathyroid hormone secretion in elderly postmenopausal women. Menopause 10: 165-71.

Welshons, W.V., M.E. Lieberman, and J. Gorski, 1984. Nuclear localization of unoccupied oestrogen receptors. Nature 307: 747-9.

Wild, P., D. Bitterli, and M. Becker, 1982. Quantitative changes of membranes in rat parathyroid cells related to variations of serum calcium. Laboratory Investigation 47: 370-4.

Wild, P., E.M. Schraner, and E. Eggenberger, 1984. Quantitative aspects of membrane shifts in rat parathyroid cells initiated by decrease in serum calcium. Biology of the Cell 50: 263-72.

Wild, P., S. Gloor, and E. Vetsch, 1985. Quantitative aspects of membrane behavior in rat parathyroid cells after depression or elevation of serum calcium. Laboratory Investigation 52: 490-6.

Wild, P., E.M. Schraner, and P. Santini-Willmes, 1989. Effects of estradiol on parathyroid cell activity. Experientia 45: 1121-3.

7 Acknowledgements

An dieser Stelle möchte ich alle danken, deren freundliche Unterstützung und tatkräftige Mithilfe zu dieser Arbeit beigetragen haben. Ohne diese Personen hätte ich die vorliegende Arbeit neben meinem beruflichen Engagement nicht zu Ende führen können. Mein besonderer Dank gilt:

Prof. Dr. P. Wild für das Überlassen des Themas, sowie seine unermüdliche und immer geduldige Art, mich mit Rat und Tat zu unterstützen. Auch war mir seine immerwährend positive Einstellung eine grosse Hilfe während des Verfassens des schriftlichen Teiles dieser Arbeit,

Prof. Dr. T. Thun für die Übernahme des Korreferats,

Frau E. M. Schraner für die technische Unterstützung,

lic. Oec. V. Couson für die wertvolle und geduldige Unterstützung bei der Statistik,

Dr. med. vet. FVH G. Stuker für das Durchlesen des Manuskripts,

meinen Mitdoktorandinnen **Hélene Leuzinger** und **Sassa Briner** für die unterhaltsamen Gespräche während und neben der Arbeit,

meinen Eltern für die liebevolle und finanzielle Unterstützung in den letzten Jahren.

8 Curriculum Vitae

Personalien	Name:	Sigrist
	Vorname:	Yvonne
	Geburtsdatum:	12. Januar 1971
	Heimatort:	Greifensee
	Nationalität:	Schweiz
	Geburtsort:	Uster
Ausbildung	1978 - 1984	Primarschule Greifensee, ZH
	1984 - 1987	Sekundarschule Nänikon - Greifensee, ZH
	1990 - 1993	Berufsschule Juventus, Arztgehilfinnenschule, Zürich
	1993 - 1995	Medizinische Laborantin Veterinaria AG, Zürich
	1995 - 1998	Medizinische Laborantin Analytika AG, Zürich
	1998 - 2001	Kantonale Maturitätsschule für Erwachsene (KME), Zürich
	2001	Eidg. Matura
	2001 - 2007	Studium der Veterinärmedizin an der Vetsuisse Fakultät Universität, Zürich
	2005	Doktorandin am Veterinär - Anatomischen Institut Vetsuisse Fakultät Universität, Zürich
	2007	Staatsexamen als Tierärztin, Vetsuisse Fakultät Universität, Zürich
	2008	Anstellung als Tierärztin, Diavet Labor AG, Bäch